Quarterly Report Q-B2375-4

# **Technical Report**

ON THE GROWTH OF CELLS IN CULTURE
(Final Report)

NSR 39-005-020

May 1, 1965 to April 30, 1966

# EFFECTS OF REPRODUCIBLE MAGNETIC FIELDS ON THE GROWTH OF CELLS IN CULTURE

This report presents the final results of studies performed in both high and very low magnetic fields. A description of the fields used was presented in a previous report (Q-B2375-3).

The data represent the combined results of all experiments performed during the course of this study. Some of these data had been presented in preliminary form in previous reports. The experiments conducted during this study are summarized as follows:

### Very low magnetic fields

Euglena gracilis

Colpidium

P. caudatum

P. multimicronucleatum

Chlorella pyrenoidosa

Clover seeds

Wheat seeds and seedlings

#### High magnetic fields

Euglena

#### P. multimicronucleatum

During the entire course of this study, as many of the environmental parameters as feasible were maintained constant. Lighting was maintained uniform by using a bank of 32 fluorescent lamps consisting of 16 40 watt cool white (F40-CWX) and 16 40 watt Gro-Lux (F40-GRO) lamps. The lighting was cycled 14

hours on and 10 hours off. Temperature was remarkably constant. The air temperature was recorded at 21°C on a Honeywell 7-day recorder. No variation was observed. The temperature of the culture medium, however, was noted daily with no daily fluctuation, but the range within the different culture jars was from 23.4° - 23.8°C. Relative humidity was not significant since liquid cultures were used; nevertheless the relative humidity of the chamber was held between 48 - 52% R.H.

With the exception of <u>Euglena</u> and <u>Chlorella</u> which were grown in Knop's solution, the other protozoa were cultured in a rice-milk medium (0.3 g powdered skim milk, 1 liter Aq. dist., 5 grains of boiled white rice).

All cell counts were made with a Model B Coulter Counter. These counts were verified by visual means using the Sedgwick-Rafter method with a Dunn counting chamber. A detailed description of the physical environment and methods used are given in a previous report (Q-B2375-3).

For the high magnetic field studies, control cultures were placed in relation to wooden blocks of the same size, color and configuration as the magnets. In the low field studies, control cultures were maintained in aluminum cylinders of the same dimensions as the high permeability metal shields. Both cylinders, experimental and control, either were painted on their internal surface with flat white paint (3 M Velvet Coating, 101-A10 white) or were lined with white paper toweling. Experimental and control cultures were set up in pairs and were run simultaneously. Any change or modification of the environment that might occur due to factors or events beyond the investigator's control would apply equally to both experimental and control cultures. In

extreme cases, experiments were discontinued and started over again. (This occurred in two instances when there was a power failure over the week end.)

### EXPERIMENTAL RESULTS

Very Low Magnetic Fields (less than one milligauss)

Fifty separate cultures of <u>Euglena gracilis</u> were grown for periods up to 21 days, with samples taken every seven days. Each sample consisted of five aliquots which were counted in the Coulter Counter and averaged. The data are shown in Table I.

Twenty-four cultures of <u>Chlorella pyrenoidosa</u> (71105) were grown for eight days in Knop's solution, with 1% CO<sub>2</sub> in air bubbled through the solution continuously at the rate of 500 ml/min. Samples were taken at the end of three, five and eight days, each sample consisting of five aliquots. Cells were counted electronically in the Model B Coulter Counter and averaged. The data are summarized in Table II.

Fifteen cultures of <u>Colpidium</u> were grown in a milk-rice medium for 21 days with samples taken at the end of seven and fourteen days. Each sample consisted of five aliquots which were counted in the Coulter Counter. Since an electrolytic medium is required in making electronic cell counts, the Coulter Counter was set and calibrated for Knop's solution as the electrolyte. In preparing the <u>Colpidium</u> samples for counting, a ten-fold concentration of one part of Knop's solution was diluted with nine parts of the <u>Colpidium</u> culture. The data obtained are presented in Table III.

TABLE I

GROWTH OF EUGLENA IN NEAR-ZERO MAGNETIC FIELDS

	Lays			† <sub>7</sub>	14 Days			77	21 Days	
<pre>Cell Count* Cells/ml</pre>	Growt	ı Ratio		Cell Count Cells/ml	Growth	Ratio		Cell Count Cells/ml	Growth	Ratio
Exp. Contl.	Exp.	Contl.		Contl.	Exp.	Contl.		Contl.	Exp.	Contl.
850 580	3.5	2.5	1920	1670	. 8.0	0.7	2460	1550	10.0	6.5
4880 3930	5.0	0•17	10100	6480	10.0	6.5	23400	27900	23.5	28.0
5980 2660	0.9	2.5	13400	10200	13.5	10.0	22800	16900	23.0	17.0
3900 2390	5.0	3.0	8470	6120	11.0	8.0	16200	15500	21.5	22.5
	Cells/ml p. Contl. 50 580 80 3930 30 2660		Growth Ratio ** Exp. Contl. 3.5 2.5 5.0 4.0 6.0 2.5 5.0 3.0	Growth Ratio***  Exp. Contl.  3.5 2.5 1  5.0 4.0 10  6.0 2.5 13  5.0 3.0 8	Growth Ratio***  Exp. Contl.  3.5 2.5 1  5.0 4.0 10  6.0 2.5 13  5.0 3.0 8	Growth Ratio***  Exp. Contl.  3.5 2.5 1  5.0 4.0 10  6.0 2.5 13  5.0 3.0 8	Growth Ratio***  Exp. Contl.  3.5 2.5 1  5.0 4.0 10  6.0 2.5 13  5.0 3.0 8	Growth Ratio ** Cells/ml Growth Fatio Exp. Contl. Exp.	Growth Ratio ** Cells/ml Growth Ratio Cells	Growth Ratio ** Cells/ml Growth Ratio Exp. Contl. Exp.

Average of eight experimental cultures and eight control cultures Average of twenty-one experimental cultures and six control cultures \* the solution

Average of twenty-one experimental cultures and seven control cultures Total average of fifty experimental cultures and twenty-one control cultures Variation in cell counts did not exceed 10%

Growth Ratio - final cell count initial inoculum

TABLE II

GROWTH OF CHLORELLA IN NEAR ZERO-MAGNETIC FIELDS

		2 Dates				5 Davs	S.			8 Days	S	
Days		) Days					)					
Initial	Cell Count*	unt*	E H-month	- *** - *******************************	Cell Count Cells/ml	Sount s/ml	Growth Ratio		Cell Cell	Cell Count Cells/ml	Growth Ratio	Ratio
Cells/ml		Contl. Exp.	3	Contl.	Exp. Contl.	Contl.	Exp.	١. ١	Exp.	Contl.	Exp.	Contl.
101001	24100	32700 2.5	2.5	3.0	25500 30400	30400	2.5	3.0	30800 26600	26600	3.0	۵. ب
6800 <sup>2</sup>	14800	15000	2.0	2.0	91.40	9140 9040	1.5	7.5	į	1	1	ı
84503	19500	19500 23900 2.0	2.0	2.5	17300	17300 19700	2.0	2.5			3.0	2. 5.

H 0. m \*

Average of 12 experimental and 6 control cultures Average of 12 experimental and 6 control cultures Total average of 24 experimental and 12 control cultures Variation in cell count did not exceed 3%

Growth Ratio = final cell count initial inoculum

TABLE III

GROWTH OF COLPIDIUM IN NEAR ZERO-MAGNETIC FIELDS

		7 Days	ys			77	14 Days			21 Days	)ays	
Initial Inoculum Cells/ml	Cell Count <sup>*</sup> Cells/ml Exp. Contl	Contl.	Growth Ratio Exp. Cont	Ratio Contl.	Cell Cell Exp.	Cell Count Cells/ml Exp. Contl.	Growth Exp.	Growth Ratio Exp. Contl.	Cell Count Cells/ml Exp. Contl.	t.	Growth Ratio Exp. Cont	Ratio Contl.
260 <sup>1</sup>	1350	1400	5.0	7.5	2030	1320	8.0	5.0	5550 2160	0	21.0	8.5
85501	0902	7800	1.0	1.0	22,100 10600	10600	2.5	7.7	7320 4990	0	8.5	0.9
2220 <sup>1+</sup>	2460	3390	1.0	1.5	2370	2860	1.0	1.5	2570 2540		1.0	1.0
1370 <sup>2</sup>	3620	3610	2.5	2.5	14300	0076	10.0	6.5	19100 10800	0	13.5	7.5
3100 <sup>3</sup>	3620 4050	4050	1,0	1.5	00011	6050	3.5	2.0	10700 5120	0	3.5	1.5

i ~ ~ \*

Average of 3 experimental cultures and 2 controls Average of 6 experimental and 2 control cultures Average of 15 experimental and 8 control cultures Variation in cell counts did not exceed 10%

Cultures failed to grow, bacterial contamination

Twenty-four cultures of Paramecium were grown on the milk-rice medium for 21 days in the very low magnetic field. These cultures were divided between Paramecium caudatum and Paramecium multimicronucleatum as follows:

P. caudatum - 12 cultures, P. multimicronucleatum - 12 cultures.

The same procedure was followed as that used with <u>Colpidium</u> (see above). The experimental results are summarized in Table IV.

A total of 3500 white clover seeds were used in experiments to determine the effect of a magnetically field-free environment on seed germination. The seeds were germinated on a weighed quantity of white, ashless filter paper pulp to which was added a measured volume of water. Three experiments were conducted: (1) germination of seeds in the experimental environment, (2) germination of seeds in experimental and control environment after one month pre-storage under experimental conditions, (3) same as (2) above but after three months pre-storage under the experimental conditions.

The germination percentage for this batch of clover seeds, as stated by the seed packer, as of July 1964 was 70% with an analysis of 98% pure white clover containing 20% hard seeds.

The experimental results are presented in Tables V, VI and VII.

As an extension of the clover seed study, 750 wheat seeds were allowed to germinate and develop to the seedling stage in the low magnetic environment. The wheat seeds were germinated and grown on a mixture of equal parts by volume of perlite, silica, and white ashless filter paper pulp. Weighed quantities of this mixture were moistened with measured amounts of tap water. After seven days, the growing shoots and roots were measured and compared with the controls. The results are summarized in Table VIII.

TABLE IV

Paramecium caudatum

	1		<del></del>			<del>,                                     </del>
	Growth Ratio	Contl.	0.5	0	0	0.5
ıys	Growth	Exp.	0.5	1.0	0	0.5 0.5
21 Days	Cell Count Cells/ml	Exp. Contl.	120	7		17
	Cell Cell	Exp.	120	12	0	717
	Growth Ratio	Contl.	3.5	0. 5.	0	3.0
14 Days	Growth	Exp.	3.0	0.5	۲.	3.0 3.0
71	<pre>Cell Count Cells/ml</pre>	Exp. Contl.	860	7	0	290
	Cell Cell	Exp.	770	2	56	270
	Growth Ratio	Contl.	0.5	о. Л	2.0	0.5
ıys	Growth	Exp.	0	0.5	2.0	0.5
7 Days	Cell Count* Cells/ml	Contl.	120	7	29	52
	Cell (	Exp.	93	2	31	144
Days	Initial Inoculum	Cells/ml	2401	161	152	903

Paramecium multimicronucleatum

1.0	0	1.0	1.0
0.5	0	1.0	0.5
190	2	58	75
130	7	31	26
1.5	0.5	2.0	1.5
ب ب	٥.5	2.5	3.0
290	11	39	110
650	12	52	540
0	0	7.0	0.5
0.5	0	3.0	0.5
30	7	79	07
63	Μ	99	777
1801	261	212	763

H 0. m \*

Average of 3 experimental cultures and 2 controls Average of 6 experimental cultures and 2 controls Average of 12 experimental and 6 control cultures Variation in cell counts did not exceed 20%

Hours	Experimental	Control
24	32%	33%
31	52%	50%
48	84%	69%

# TABLE VI

Percent Seed Germination after 1 Month Pre-Storage in a "Zero" Magnetic Field (1500 seeds)

	Exper	imental	Cont	rol
Time in Hours	Al	E <sup>2</sup>	Al	E <sup>2</sup>
24	35%	17%	32%	19%
31	53%	32%	51%	30%
48	89%	74%	69%	67%

- 1. A seeds stored under ambient conditions
- 2. E seeds stored under experimental conditions

# TABLE VII

Percent Seed Germination after 3 Months Pre-Storage in a "Zero"

Magnetic Field (1250 seeds)

	Experi	mental	Contr	ol
Time in Hours	Al	E <sup>2</sup>	Al	E <sup>2</sup>
24	29%	27%	35%	34%
31	51%	41%	49%	50%
48	79%	62%	69%	69%

- 1. A seeds stored under ambient conditions
- 2. E seeds stored under experimental conditions

TABLE VIII

Growth of Wheat Seeds (750 seeds) after One Week Exposure to a

"Zero"	Magnetic	Field
--------	----------	-------

	Percentage		Growth		Growth
	<u>Germination</u>	Root Length <sup>2</sup>	Difference <sup>2</sup>	Shoot Length <sup>2</sup>	<u>Difference</u> <sup>2</sup>
Experimental	78%	42 <u>+</u> 12 mm		73 ± 31 mm	
			5 ± 3 mm		8 ± 5 mm
Control	79%	37 ± 11 mm		65 ± 30 mm	

- 1. Based on 750 experimental and 750 control seeds
- 2. These values represent the mean of the parameter under consideration with the variation representing the average difference from the mean.

### High Magnetic Fields

The fields used in this portion of the study were generated by large permanent magnets (surplus magnetron magnets). Various gap distances were used in the several magnets to give the desired field strengths. These may be listed as follows:

Magnet	Field Strength
1	400 - 800 oersteds
lA	400 - 800 "
2	550 -1100 "
2A	90 - 120 "
3	250 - 375 "
3 <b>A</b>	550 -1100 "
4	250 - 400 "
LμA	100 - 125 "
5	80 - 100 "
5A	80 - 90 "
6	90 - 120 "
6A	400 - 800 "

In the magnetic field studies, each experimental configuration consisted of a magnet and its control. In each case the control was a wooden dummy of the magnet, same size, shape, gap, color, etc. The magnet and its control were placed under the same bank of lights and each experimental-control pair was treated as a unit.

Euglena gracilis cultures were exposed to six different magnetic fields continuously for 28 days, samples were taken every seven days and counted on the Coulter counter. Four such replicates were made over a 4-month period. The resultant data are summarized in Table IX.

The effect of exposure to magnetic fields greater than ambient as compared to controls is summarized in Table X. These data represent the total average of all experiments in all magnetic fields (combined).

A similar series of experiments was performed over a 21 day period using Paramecium multimicronucleatum. Six additional magnets were used, but each was of the same field intensity, gap dimensions, and configuration as those used in the Euglena experiments. This series was repeated twice over a 2-month period. The resulting data are summarized in Table XI.

## DISCUSSION

In examining the data, it may be noted that four cell-systems were used in the low field studies. These included <u>Euglena</u>, <u>Colpidium</u>, <u>Paramecium</u>, and <u>Chlorella</u>. This choice represents two simple animals and two simple plants of different cell size ranges.

This cell size range is represented as follows:

Table IX

Growth of Euglena in Magnetic Fields Greater than Ambient

	ή	<del></del>	+					
	wth	Control	12.0	15.0	14.5	14.0	15.5	12.5
days	Growth Ratio	Experiment	11.0	12.5	11.0	13.5	13.5	13.5
28	Cell Count Cells/ml	Control	12900	15800	15500	006ητ	16300	13100
	Cell Cell	Experiment	11800	13300	11900	14200	14200	14300
	wth	Control	8.0	0.6	9.0	9.0	9.5	9.5
Days	Growth Ratio	fremireqx	8.0	8.0	8.0	0.6	8.5	8.0
21	Cell Count Cells/ml	Control	8370	0956	9380	9370	10100	10200
	Cell Cel	Experiment	8520	8500	8170	9310	8980	8690
	Growth Ratio	Control	7.0	5.0	0.4	5.5	5.0	5.0
14 Days		Experiment	4.5	4.0	0.4	4.5	4.5	4.0
777	ell Count Cells/ml	Control	4360	5390	4520	5770	5340	5280
	Cell Cel	Fxperiment	00.27	4270	0644	7,860	7630	14420
	Growth Ratio	Control	3.5	3.0	3.0	3.0	3.0	3.0
ys		Experiment	3.0	3.0	3.0	3.0	3.0	3.0
7 Days	Cell Count Cells/ml	Control	3740	3460	3240	3380	3320	3350
	Cell Cell	framiragx	3350	2960	3320	3130	3020	2930
ys	слŢтш	onl <sup>[</sup> [sitin] fm\sf[90]	1060	1060	1060	1060	1060	1060
Days	J	Magnet numbe	A.	প্র	33	3	A <sub>I</sub> I	7

1. Average of 24 cultures, covering six different magnetic field intensities

TABLE X

Combined Data Representing Total Average of All Experiments in All Magnetic Fields

	rth .o	Control	12.5 14.0
Days	Growth Ratio	Experiment	
28 1	ount	Control	13300 14800
	Cell Count	Fxperiment	13300
	th o	Control	8.0 9.0
21 Days	Growth Ratio	Experiment	8.0
21	Cell Count	Control	8700 9500
	Ce11	Experiment	8700
3	Growth Ratio	LortroD	0.5 0.4
Lt Days	Gro Rat	Experiment	7.0
1	Cell Count	Lortnol	5110
	Cell	Experiment	7,560
	th o	Control	3.0
ys	Growth Ratio	fremireqxA	3.0
7 Days	Cell Count	Lortnol	.060 3120 3420 3.0 3.0
	Ce11	fremireqxA	3120
Days		Initial muculum	1060

1. Average of  $2\mu$  cultures, covering six different magnetic field intensities

TABLE XI

Growth of P. multimicronucleatum in Magnetic Fields Greater than Ambient

	io	Contl.				<u> </u>			
	h Rat	Con					· · · · · · · · · · · · · · · · · · ·	<del></del>	1.0
21 Days	Growth Ratio	Exp.	1.0	0	0.5	1.0	0	0	0.5
27	Cell Count Cells/ml	Exp. Contl.			<del></del>				110
	Cell Cell		80	56	37	75	32	57	917
	Ratio	ontl.	•					<del></del> >	1.0
14 Days	Growth Ratio	Exp. Contl.	2.0	2.0	2.0	2.0	1.5	1.5	2.0
17	ount /ml	ontl.						<del>)</del>	85
	<pre>Cell Count Cells/ml</pre>	Exp. Contl.	170	160	170	170	130	140	160
	atio	ontl.						<del>-</del>	3.0
ys	Growth Ratio	Exp. Contl.	1.0	1.5	3.4	1.0	0.5	0.5	1.5
7 Days	unt ml	Contl. <sup>2</sup>						<del>&gt;</del>	230
	Cell Count Cells/ml	Exp. 1	100	120	270	98	99	58	120
	Initial	Number Cells/ml	81	81	81	81	81	81	81
Days	70	Magner Number	гH	α	77	ξĀ	9	6A	Combined Average

5.5

Average of two cultures for each magnet Separate controls did not accompany each magnet. Each experiment consisted of six magnets and two controls. This figure represents the average of the four controls (two replicates of two each).

Euglena - 50 - 100 microns

Chlorella - 20 - 30 microns

Colpidium - 50 - 100 microns

Paramecium 500 - 1000 microns

The original intent was to determine whether cell size has any relationship to the biological effect induced by the very low magnetic field and whether any cell-size differences could be observed between experimental and control cultures after exposure to the low field.

Biological effects that are represented by the reduced data presented in the previous section will be discussed below. However, at this time, any differences in cell size between control and experimental cultures may be dispensed with by noting that none were observed. At the time cell counts were made, cell size distribution was automatically plotted (using the Model B Coulter Counter and Automatic Size Distribution Plotter). In no case could any difference be detected for cell size and size distribution plot between the experimental and control cultures.

Examination of the low magnetic field data shows a response in the same direction for the various cell cultures used. This response is summarized in Table XII.

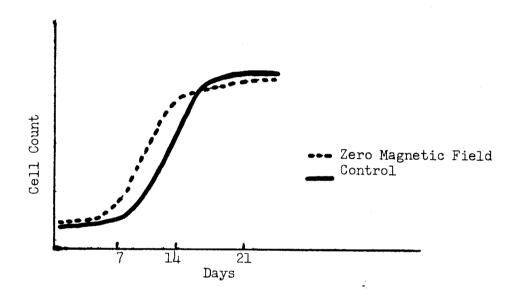
The cultures of <u>Euglena</u> showed a definite increase in growth rate when maintained under conditions where the magnetic field is lacking. This increased growth rate, as compared to control cultures, continued through 14 days, but then the growth rate of controls increased so that by 21 days both the experimental and control groups showed essentially similar growth ratios. This would seem to indicate that the logarithmic phase of the growth curve is reached sooner

TABLE XII

Growth Response of Organisms to a "Zero" Magnetic Field

Days		7	Days	14	Days	21 Day	<i>y</i> s
	Cell Size in		h Ratio		h Ratio		n Ratio
Organism	Microns	Exp.	Contl.	Exp.	Contl.	Exp.	Contl.
Euglena	75	5.0	3.0	11.0	8.0	21.5	22.5
Colpidium	75	1.0	1.5	3•5	2.0	3•5	1.5
P. caudatum	750	0.5	0.5	3.0	3.0	0.5	0.5
P. multimicro-	750	0.5	0.5	3.0	3.0	0.5	0.5
		3	Days	5	Days	8 Daj	7S
Chlorella	25	2.0	2.5	2.0	2.5	3.0	2.5

in the very low magnetic field than it is under control conditions. The following graph indicates a possible growth reaction of cells in the very low field.



A similar pattern of effect has been observed in cultures of Colpidium. It is interesting to note, however, that both Euglena and Colpidium are about the same size organism. Also, it should be pointed out that the growth requirements are different for these two organisms: Euglena may be classified as a plant since it contains chlorophyll and photosynthesizes whereas Colpidium is an animal deriving its growth requirements from its nutrient medium.

Both species of Paramecium showed the same response. Population size decreased from the original inoculum during the first week. After the culture became established, growth progressed during the second week, and as the nutrients in the medium became exhausted and metabolic end products accumulated, growth diminished again in the third week. The same growth ratios occurred in experimental and control groups indicating that, in this instance, the environmental differences had no effect. A similar type of response was observed in the

algae, <u>Chlorella</u>, over a period of eight days. During this period no growth differences were observed. Perhaps, if the cultures of <u>Chlorella</u> were continued for 21 days an effect might have been seen.

The failure of <u>Paramecium</u> to follow the patterns established by Euglena and Colpidium might be due to two possible factors:

- (1) There is a cell-size sensitivity which affects the response to the magnetic environment.
- (2) Other factors in the environment beyond the range of experimental control.

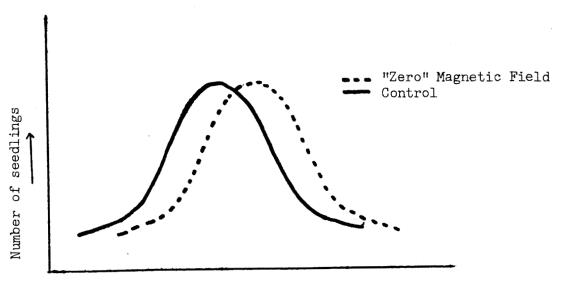
Although this first premise looks inviting, it appears unlikely in light of the results obtained with the clover and wheat seeds. After 48 hours, a higher percentage of clover seeds germinated in the "zero" magnetic field than in the ambient. Again this seems to indicate that the developmental process is apparently accelerated in the absence of a magnetic field. Tables VI and VII present data on the effect of pre-storage of seeds in the very low magnetic field prior to germination. Those seeds pre-stored in the "zero" magnetic environment for one month and then subsequently germinated in the same environment showed a lower percentage germination (74%) than those seeds not pre-stored (ambient stored seeds), then germinated in the low magnetic environment (89%). When germinated under control conditions, pre-storage made no difference in percent germination (69% not pre-stored vs. 67% pre-stored). Similarly pre-storage for three months made no difference in germination in the control environment (Table VII). However, in the low magnetic field, the prestored seeds had a lower percentage germinating than in the control environment, and still less than the number of ambient stored seeds germinating in the magnetic-field free environment (Table VII). These results seem to indicate that

pre-storage of seeds has little consequence upon subsequent germination.

This might be expected since a plant seed is in a dormant state and therefore tends to react more slowly, if at all, to its environment. (Conditions of humidity and temperature which are important in maintaining seed viability during storage are not being considered since these have been maintained constant.)

Because of the noted differences in numbers of seeds germinating, further studies were initiated to determine the low magnetic field effects upon the growth of seedlings. For this purpose wheat was used and wheat seeds germinated and seedlings maintained for seven days under the conditions of the experiment. After seven days, no difference in germination percentage between experimental and control batches was observed.

A consistent difference was observed in the length of the growing shoots and roots of the experimental plants compared to controls. The seedlings grown in the low magnetic field were larger and more robust than the controls. The differences noted may be graphically demonstrated in the following curves which represent the size distribution of the seedlings.



Length in Millimeters

These curves show a similar displacement toward greater growth in the very low magnetic field grown seedlings as was seen in the growth curves for Euglena.

From these data, there is more than just a general trend toward growth enhancement in the very low fields. In those cases where close control was exercised over the environmental variables (within limits of feasibility), a reproducible pattern of increased growth in very low magnetic fields (less than 1 milligauss) was observed. However, these data are considered inconclusive at this time because exposure was of a relatively short duration. In none of our previous studies have we ever found an effect due to acute exposure. This lack of effect in short term experiments had been previously reported for tissue culture cell lines. In order to get the full impact of the low magnetic field effect, plant studies must be done over a time period long enough to allow the plants to mature and develop seeds and then these seeds germinated and grown in the very low magnetic field. However, it should be pointed out that when a size distribution of the wheat seedlings was made, the resultant plot was a bimodal curve indicating that these wheat seeds were not from a homogeneous population. This heterogeneity may explain the failure to get similar data to that obtained from the clover seed study.

Examination of the data obtained from magnetic fields greater than ambient, results in a more complex situation. Complicating factors are introduced by differences in magnetic field strength and gradients within the field (field homogeneity).

TABLE XIII

Growth Response of Euglena to Magnetic Fields Greater than Ambient

Days		7	7 Days	ľ	14 Days	7	21 Days	35	28 Days
Magnetic Field	ield	Growt.	Growth Ratio	Growt	Growth Ratio	Growt	Growth Ratio	Growt	Growth Ratio
Strength (Oersteds)	rsteds)	Exp.	Control	Exp.	Control	•dx <sub>E</sub>	Control	Exp•	Control
700 - 800	AL	3.0	3.5	4.5	0.4	8.0	8.0	11.0	i2.0
90 - 120	2A	3.0	3•0	0.4	5.0	8.0	0.6	12.5	15.0
550 - 1100	3A	3.0	3.0	70.1	0•17	8.0	0.6	0.11	14.5
250 - 375	3	3.0	3.0	4.5	5.5	9.0	0.6	13.5	14.0
100 - 125	PT-	3.0	3.0	4.5	5.0	8 5.5	9.5	13.5	15.5
80 - 100	Ŋ	3.0	3.0	0.4	5.0	8.0	9.5	13.5	12.5
					ļ				

Referring to Table X, examination of the growth ratios indicates that magnetic fields greater than ambient tend to have an inhibiting effect upon the population growth of cultures of <u>Euglena</u> over a period of 28 days. However, examining the data in terms of field strength and homogeneity, a similar pattern of effect still emerges but with some interesting overtones. These data are summarized and presented in Table XIII.

Practically no effect was observed for magnets 1A and 3 which represent fields of 400 - 800 and of 250 - 375 oersteds respectively. The greatest effect was seen for magnets 2A, 3A, 4A, and 5 which represent fields of 90 -120, 550 - 1100, 100 - 125, and 80 - 100 oersteds respectively. However, these various field intensities represent a rather wide range of gradients. Contour plots of these magnetic fields were drawn and presented in a previous report (Q-B2375-3). For convenience at this time, the magnetic field intensities and their gradients are summarized below:

Magnet No.	Field Intensity	Field Gradient
1	400 - 800 oersteds	30 - 70 oersteds/cm
lA	400 - 800 oersteds	40 - 70 oersteds/cm
2	550 - 1100 oersteds	50 - 80 oersteds/cm
2 <b>A</b>	90 - 120 oersteds	3 - 4 oersteds/cm
3	250 - 375 oersteds	10 - 18 oersteds/cm
3 <b>A</b>	550 - 1100 oersteds	50 - 90 oersteds/cm
14	250 - 400 oersteds	10 - 25 oersteds/cm
λt	100 - 125 oersteds	2 - 6 oersteds/cm
5	80 - 100 oersteds	l - 2 oersteds/cm
5 <b>A</b>	80 - 90 oersteds	l - 2 oersteds/cm
6	90 - 120 oersteds	2 - 4 oersteds/cm
6A	400 - 800 oersteds	25 - 75 oersteds/cm

With the exception of magnet 3A which represents the highest field intensity used, the magnetic fields that showed the greatest biological response were those with the most homogeneity (Magnet 2A, 90 - 120 oersteds, gradient 3 - 4 oersteds/cm; Magnet 4A, 100 - 125 oersteds, gradient 2 - 6 oersteds/cm; Magnet 5, 80 - 100 oersteds, gradient 1 - 2 oersteds/cm). Is there a relationship between field homogeneity and biological response? Based on these data, no definite conclusions can be drawn. The response to the magnetic fields of various field strengths and various gradients are less clear cut and more difficult to explain than the effects observed in the very low fields.

Nevertheless, if the combined magnetic field data are used (Table X), then

there is a clear indication of growth inhibition in higher than ambient magnetic fields.

For purposes of discussion, the growth ratios for cultures of <u>Euglena</u> exposed to high and very low magnetic fields are compared in Table XIV (data from Table X and Table XII).

These data may be represented pictorially in the following graph.

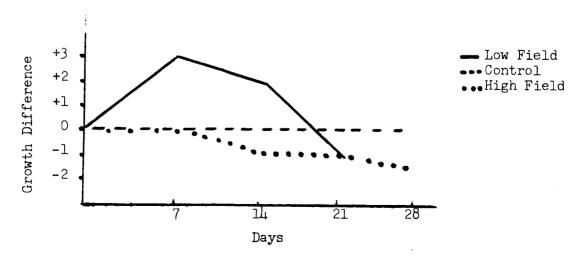


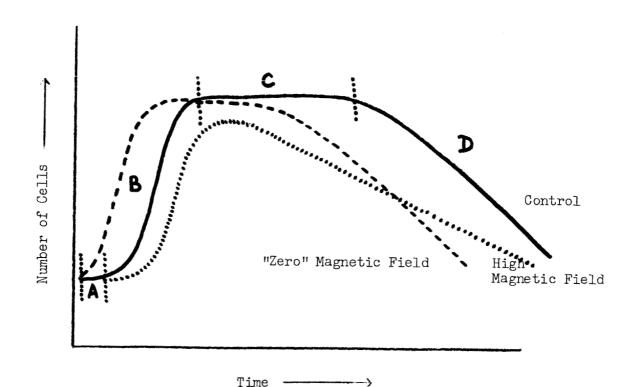
TABLE XIV

Response of Euglena to High and Very Low Magnetic Fields

		<u> </u>						
Days	7 ]	7 Days	ήT	114 Days	21	21 Days	28 Days	ays
	Growt	Growth Ratio	Growth	Growth Ratio	Growt	Growth Ratio	Growth	Growth Ratio
Magnetic Field	Exp.	Control	Exp.	Control	Exp.	Control	Exp.	Control
Very Low	5.0	3.0	0,11	8.0	21.5	22.5		t
Higher than Ambient	3.0	3.0	0.4	5.0	8.0	0.6	12.5	14.0
	Gr. Diff	Growth Difference	Grc Diffe	Growth Difference	Growth Difference	wth rence	Growth Difference	rth ence
Very Low	+	+ 2.0	+ 3.0	0.0	- 1.0	0.	1	
Higher than Ambient		0.0	- 1.0	0	- 1.0	0.	1	- 1.5

Growth Difference = Experimental Growth Ratio - Control Growth Ratio Sign indicates acceleration of growth in the experimental culture over the control. Sign indicates inhibition of growth in the experimental cultures over the control. i + 1

This graph may be interpreted by saying that removal of the normal magnetic field results in a sudden increase in growth activity which may be the result of either the elimination of the lag phase of a normal growth curve or a time shift of the growth curve so that the logarithmic phase occurs earlier. The stationary phase may be abbreviated with the death phase occurring sooner. Thus by 21 days, the population of <a href="Euglena">Euglena</a> in the very low magnetic field shows a lower growth ratio than the controls. On the other hand, in a higher than ambient magnetic field, the growth curve proceeds at a normal rate, but since there is an inhibition of growth after seven days, it might be conjectured that the logarithmic phase of growth is shortened, the stationary phase begins earlier and is abbreviated with a slowly developing death stage ensuing. This entire process may be schematically represented in the following diagram.



A = lag phase of growth

B = logarithmic phase of growth

C = stationary phase

D = death phase

This explanation of the results reconciles the differences observed between "zero" magnetic field and control cultures and between higher than ambient magnetic field and control cultures. Unfortunately, however, based on these data, no mechanistic explanation may be offered at this time. To relate these results to a functional process would require another series of studies probing the biochemical and biophysical properties of the cells under the experimental environmental conditions.

## SUMMARY

A series of biological specimens, both plant and animal, simple and complex, have been exposed to magnetic fields of varying intensities varying from near zero (less than one milligauss) to higher than ambient (1100 oersteds maximum). The general effect seems to be one of growth acceleration in the very low field; in the higher than ambient fields, there is an indication of growth inhibition. The high magnetic field results compare favorably with those reported by other investigators. Since this is the first study done in the near "zero" field, the results are interesting in that growth acceleration seems to be induced as a result of relatively long-term exposure. A possible explanation for these effects is presented.

M. H. Halpern, Ph. I. Principal Life Scientist Bio-Instrumentation Lab

Approved by:

R. M. Goodhan, Manager Bio-Instrumentation Lab C. W. Hargens, Mechnical Director Electrical Engineering Division